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Chemical Characterization and Evaluation of the Antimicrobial Properties of Leaf Extracts from Five Ethnomedicinal Plants in Paschim Medinipur District, West Bengal, India

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Abstract: This study examines the physicochemical properties, phytochemical composition, and antimicrobial potential of five medicinal plants—*Acalypha indica*, *Senna tora*, *Euphorbia hirta*, *Physalis angulata*, and *Ziziphus mauritiana*—traditionally used in folk medicine. Fresh leaves were collected from Paschim Medinipur district, West Bengal, India and subjected to proximate and ultimate analyses, Fourier-transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS), and antimicrobial assays against *Escherichia coli* and *Staphylococcus aureus*. Results revealed that *Acalypha indica* exhibited the highest levels of volatile matter (75.452%), cellulose (61.727%), and tannins (0.635 g/100g), indicating a rich phytochemical profile. *Physalis angulata* recorded the highest fixed carbon (15.251%) and extractive content (1.974%), while *Senna tora* showed elevated ash (12.327%) and lignin content (5.531%). *Euphorbia hirta* had the highest carbohydrate (9.619 g/100g) and nitrogen content (2.050%) and *Ziziphus mauritiana* displayed the highest protein (2.321 g/100g), fat (0.655 g/100g) and oxygen content (34.222%). FTIR confirmed the presence of key functional groups, and GC-MS identified 10 bioactive compounds in *A. indica* and *S. tora* and 8 in the remaining plants. Several compounds, such as Cyclotrisiloxane, Myo-Inositol, and 4-Tert-Octylphenol, lacked prior records of individual bioactivity and are considered novel, warranting further investigation. Antimicrobial tests showed strong activity against *Staphylococcus aureus*, a Gram-positive bacterium, but no inhibition of *Escherichia coli*, likely due to its outer lipopolysaccharide membrane acting as a barrier to bioactive compounds. The results of this study demonstrate that plant extracts from *Acalypha indica*, *Senna tora*, *Physalis angulata*, *Euphorbia hirta* and *Ziziphus mauritiana* exhibit significant antibacterial activity against *Staphylococcus aureus*, a Gram-positive bacterium, while showing no inhibitory effect against *Escherichia coli*, a Gram-negative bacterium. The susceptibility of *S. aureus* to plant extracts, as indicated by the MIC, MBC, and DAD assays, strongly suggests that these extracts contain bioactive compounds capable of penetrating and disrupting bacterial cell structures. However, the absence of antimicrobial activity against *E. coli* supports the well-established concept that Gram-negative bacteria have an outer membrane composed of lipopolysaccharides (LPS), which acts as a selective permeability barrier, preventing many antimicrobial agents from entering the cell. The study validates the traditional medicinal use of these plants and highlights their potential as sources of novel antibacterial agents and phytopharmaceuticals.

Introduction

Medicinal plants are highly regarded for their bioactive compounds, which are natural substances that can impact human health in diverse ways. These include secondary metabolites, which are not directly involved in essential processes such as growth or reproduction but are important for plant defense against pests and pathogens, attracting pollinators, and coping with environmental stressors (Maiti et al., 2010, 2013; Banerjee et al., 2014; Eljounaidi and Lichman, 2020; Acharya et al., 2022). Bioactive compounds like alkaloids, flavonoids, terpenoids, and phenolic compounds are distributed

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throughout different parts of the plant, with leaves being one of the common sites where they are found.

The concentration of secondary metabolites can vary due to several factors, including climate change. Elements such as rising temperatures, elevated carbon dioxide (CO_2) levels and shifts in precipitation patterns can all influence their levels in plants. Edaphic factors refer to the soil-related factors such as soil composition, pH, moisture content, and nutrient availability. Altitudinal variation, which refers to changes in elevation or altitude above sea level, can significantly impact plant secondary metabolites. As plants grow at different altitudes, they experience variations in environmental factors such as temperature, precipitation, light intensity, atmospheric pressure, and oxygen levels. These changes can influence the production and composition of secondary metabolites (Ma et al., 2020). So, the chemical characterization of medicinal plants is urgently needed today to find actual bioactive phyto-constituent used by indigenous people (Acharya, 2015; Acharya, 2016; Acharya et al., 2021a,b; Acharya et al., 2022; Sarkar and Madhu, 2017; Sarkar et al., 2021; Sarkar et al., 2022; Alam et al., 2022; Antony et al., 2018; Roy et al., 2023; Sarkar et al., 2023).

The chemical characterization of leaves from medicinal plants involves identifying and quantifying various chemical compounds present in the leaves. Medicinal plant leaves can contain a wide range of bioactive compounds and understanding their chemical composition is essential for assessing their potential therapeutic properties.

Some common methods and techniques used for the chemical characterization of medicinal plant leaves include: Extraction of Bioactive Compounds using appropriate solvents like ethanol, methanol, water, or a mixture of solvents, Qualitative and Quantitative Analysis through thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and Mass spectrometry is used for the determination of molecular weights and the identification of compounds based on their mass spectra gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) etc.

Paschim Medinipur (West Midnapore), located in the Indian state of West Bengal, is rich in biodiversity and has a long history of traditional herbal medicine practices. The region is home to various indigenous communities that have relied on the abundant flora for their medicinal needs.

In the present investigation, five potential medicinal plants viz., *Acalypha indica*, *Senna tora*, *Euphorbia hirta*, *Physalis angulata* and *Ziziphus mauritiana,* have been collected from Paschim Medinipur district of West Bengal, India and screened for their potential bioactive phytochemicals and their antimicrobial activities.

Acalypha indica, commonly known as Indian copperleaf or Indian nettle, is a member of the Euphorbiaceae family. It is a perennial herb that typically grows to a height of 30-80 centimetres. It is native to India and is found in various parts of South Asia, Southeast Asia, and other tropical and subtropical regions. This plant is known for its various traditional and medicinal uses. It has simple, alternate leaves that are typically green, with serrated edges. *Acalypha indica* has a long history of traditional medicinal uses in various cultures. It is believed to have anti-inflammatory, analgesic, and antipyretic (fever-reducing) properties. Traditional medicine has been used to treat conditions like skin disorders, wounds, diarrhea, and as a pain reliever. The plant contains various phytochemicals, including alkaloids, flavonoids, tannins, and terpenoids, which are believed to contribute to its medicinal properties (Mondal et al., 2021; Islam et al., 2019).

Senna tora, commonly known as sickle senna, sicklepod, or coffee senna, is a plant species belonging to the Fabaceae family. It is an annual or short-lived perennial herb that can grow to a height of 30-90 centimeters. It is native to many tropical and subtropical regions, including parts of Asia, Africa, and the Americas. It has pinnately compound leaves with oblong leaflets. *Senna tora* has a history of traditional medicinal uses in various cultures. It is believed to have laxative, purgative, and anthelmintic (worm-expelling) properties. In traditional medicine systems like Ayurveda, the plant has been used to treat constipation, skin diseases, and other ailments. The seeds are often used in traditional remedies. *Senna tora* leaves contain several bioactive compounds, including anthraquinones, flavonoids and other phytochemicals responsible for their medicinal properties.

Euphorbia hirta, commonly known as asthma weed, garden spurge, or hairy spurge, is a small, herbaceous plant belonging to the Euphorbiaceae family. It is a low-growing, prostrate or erect annual herb typically reaches a height of 20-50 centimeters. It is native to various parts of the world, including tropical and subtropical regions of Asia, Africa, the Americas, and the Pacific Islands. The plant is covered with fine, soft hairs, which give it a somewhat fuzzy appearance. The leaves are opposite and oblong to lanceolate, with serrated edges. It is used as a remedy for a wide range of ailments, including respiratory conditions like asthma, bronchitis, and coughs

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(Jain et al., 2021). It is also used for digestive issues, diarrhea, dysentery and skin conditions like wounds and rashes (Iskandar et al., 2021).

The plant contains various phytochemicals, including alkaloids, flavonoids, tannins, and terpenoids, which are believed to contribute to its medicinal properties (Ezikanyi and Linian, 2021; Islam et al., 2019).

Physalis angulata, commonly known as hairy ground cherry, is a small annual herbaceous plant belonging to the Solanaceae family. It is a low-growing plant, typically reaching heights of 30-60 centimeters. This plant is native to tropical regions of the Americas but has become naturalized in various parts of the world due to its adaptability and ability to spread. The leaves are ovate to lanceolate, with serrated edges. In some traditional herbal medicine systems, various parts of the plant, including its leaves and fruit, are utilized for their potential medicinal properties. They are believed to have anti-inflammatory, diuretic, and antimicrobial properties. However, it's essential to exercise caution and consult with a healthcare professional before using any plant for medicinal purposes. The plant contains various chemical compounds such as Alkaloids, Flavonoids, Steroids, Terpenoids, Phenolic Compounds bearing potential medicinal values.

Ziziphus mauritiana, commonly known as Indian jujube, is a small deciduous tree or shrub belonging to the family Rhamnaceae. It is a small to medium-sized shrub or tree that typically reaches heights of 2 to 8 meters. It is native to various parts of Asia, the Middle East, and Africa. This plant is known for its edible fruits and has several other traditional and ecological uses. It has a bushy and often spiny appearance, with glossy green leaves that are ovate to elliptical in shape. In traditional herbal medicine, various parts of the plant have been used to treat ailments such as digestive disorders, coughs, and colds. The fruit is sometimes used to alleviate stomach discomfort. The chemical composition of leaves can vary depending on factors such as the plant's age, environmental conditions, and geographical location. However, some common classes of compounds found in the leaves include Phenolic Compounds, Alkaloids, Triterpenoids, Saponins, Volatile Compounds etc.

Material and Methods

Study area

Paschim Medinipur, also known as West Midnapore, is a district in the southwestern part of West Bengal, India. It is characterized by diverse geography, including forests, rivers, and agricultural land. The district shares its boundaries with the West Bengal districts of Bankura, Purulia, Jhargram and Paschim Bardhaman. Its geographic coordinates range from 21.470°N to 23.000°N in latitude and 86.400°E to 87.520°E in longitude. To the south, the district borders the Bay of Bengal coastline.

Temperature

A tropical climate is characterized by hot and humid summers, a monsoon season, and relatively mild winters. Summers, which last from March to June, can be quite hot, with temperatures frequently rising above 30°C (86°F), with April and May usually being the hottest months. Winters, which occur from December to February, are generally more moderate, with temperatures typically ranging between 15°C and 25°C (59°F to 77°F).

Climate

Paschim Medinipur experiences a monsoon season from June to September, receiving substantial rainfall during this period. The southwest monsoon brings heavy rains that are vital for agriculture but can sometimes cause flooding in certain areas. The post-monsoon months of October and November are typically pleasant, featuring cooler temperatures and lower humidity. Additionally, the district encompasses forested regions that significantly influence its climate and ecology.

Collection of samples and authentication

The plant was collected from the Paschim Medinipur district of West Bengal, India, and identified and authenticated using the book *Bengal Plants* by David Prain. Following collection, the plant was prepared for the herbarium using standard procedures. The herbarium sheet has been deposited at the Department of Botany, Belda College, Paschim Medinipur, West Bengal. Mature fresh leaf samples of five selected medicinal plants were gathered from the study area and stored separately in sterilized polythene bags, each labeled with the respective plant’s name. To preserve the chemical integrity of the leaves, they were carefully air-dried and then stored in airtight containers kept in a cool, dark place to prevent degradation. The dried leaves were subsequently ground into a powder with a minimum particle size of 1 mm.

Physical analysis

Proximate Analysis

Proximate composition analysis was conducted using standard test methods: ash content was measured following the ASTM E1755 procedure for ash in biomass, while volatile matter was analyzed according to the standard test method for volatile matter in particulate wood fuels [E 872–82 (Reapproved 1998)]. Fixed carbon content was calculated by difference.

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Bulk density and Swelling index

Bulk density was determined using the Standard Test Method for Specific Gravity of Solids (ASTM D854-92), while the swelling index was measured following ASTM D4829-11.

Compositional analysis

Compositional analysis was conducted following the standards set by ASTM International (2015) and the methodology described by Van Soest et al. (1991).

Chemical and spectroscopic analysis

Preparation of Extract: The extract was prepared using a Soxhlet apparatus, where an 8-hour methanol extraction was carried out. Afterwards, the extract was concentrated by evaporation under reduced pressure using a rotary vacuum evaporator.

Qualitative Phytochemical Studies

Preliminary Phytochemical Screening

Qualitative phytochemical tests were conducted (Balamurugan et al., 2019; Ogbuagu et al., 2020; Junaid and Patil, 2020; Sahira Banu and Cathrine, 2015; Silva, GO et al., 2017; Singh and Kumar, 2017; Nazril et al., 2016) to identify the presence of active phytochemical components such as alkaloids, proteins, carbohydrates, total phenolic compounds, tannins, fats, and others. The procedure was carried out as follows:

Test for carbohydrates

A. Benedict's test

The Benedict's reagent was added to approximately 0.5 ml of the extracted solution. The mixture was cooked for about 2 minutes in a bath of boiling water. As a result, a red precipitate emerges, indicating that sugar is present.

B. Molisch's test

2 ml of the solution was combined and shaken with 2 drops of the napthol alcohol solution. Later, 2-3 drops of concentrated H_2SO_4 were dropped into the test tube's side wall. The appearance of a violet ring indicates the presence of sugar, which can detect proteins.

Test for protein

A. Biuret test

To around 2 ml of the extracted solution, a drop of 2% copper sulphate solution was added. Then, 1 ml of 95% ethanol was added. Next, KOH pellets were added. A pink solution formed in the ethanolic layer, suggesting the presence of protein.

B. Xanthoproteic test

Two millilitres of the extracted solution were combined with a few drops of concentrated nitric acid (HNO_3) to produce a yellowish output. A protein is present because of its colour.

Test for fat

A. Saponification test

About 2 ml of the extracted solution was mixed with a small amount of 0.5N alcoholic KOH and a small amount of phenolphthalein. The mixture was heated for around two hours. There are indications that fixed oils or fats are present in the sample when soap is produced or when alkali is partially neutralised.

B. Spot test

An oil stain resulted from applying a small amount of the extract to one filter paper and pressing it with a different filter paper. It is obvious that solidified oil is present there.

Test for alkaloid

A. Wagner's Test (Potassium iodide)

In the test tube holding 500 ml of the extract solution, 1–2 drops of Wagner's reagent (1.27g I2 + 2g KI in 100 ml distilled water) were applied along the side. For 30 minutes, the combinations were heated at 60°C. Alkaloids can be detected using Wagner's reagent by looking for a reddish-brown precipitate.

B. Dragendroff's Kraut's test

The extracted solution was mixed with 1-2 drops of Dragendroff's reagent (10 ml stock solution, 20 ml acetic acid and distilled water). A reddish-brown precipitate emerges as a result, indicating the presence of an alkaloid.

Test for phenols

A. Ferric Chloride Test

An appropriate mixture was made with 1 ml of the extracted solution, 1 ml of ethanol, and a few drops of FeCl3. The plant extracts revealed blue, green, purple, and red phenolic chemicals.

B. Ellagic Acid Test

5% glacial acetic acid and then 5% sodium nitrite solution were added to 2 ml of the aqueous extract solution. The presence of a muddy solution or Niger brown precipitation confirmed the presence of phenols.

Tests for tannins

A. 10% NaOH test

0.4 ml of plant extraction solution was added to 4 ml of 10% NaOH solution, which was then violently agitated for a time. The development of an emulsion indicated the existence of hydrolysable tannins.

B. Gelatin test

5 ml of distilled water is used to dilute the plant extract. Later, 1% gelatin solution and 10% sodium chloride were added. Because of the tannin, the precipitate turns white.

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Quantitative Phytochemical studies

Estimation of the Value of Carbohydrates

The anthrone technique was used to estimate the quantity of carbohydrates. 4ml of anthrone reagent was added to 1ml of sample. After 8 minutes of incubation in a boiling water bath, the absorbance was measured at 630 nm and compared to a blank for the reagent. The results were represented as mg/gdw sample after the estimate was carried out in triplicate.

Estimation of Value of Protein

Using the Lowry technique, protein content was estimated. Two millilitres of the sample were obtained, five millilitres of newly made alkaline copper reagent were added, and after 10 minutes at room temperature, Folin-Ciocalteau reagent was added. Incubate at room temperature for 30 minutes in the dark after thoroughly mixing. Additionally, a reagent blank was originated. The blue colour that was displayed was read at 660 nm.

Estimation of the Value of Fat

The test samples were dried and ground into powder, and 100 mg of the mixture was added to a conical flask with 30 ml of chloroform and methanol (2/1:v/v) before being transferred. This mixture was well combined before being incubated at room temperature in the dark for the whole extraction process. Then, 20 ml more chloroform and 2 ml of water were added and centrifuged to separate the two layers. All of the lipids (in chloroform) were present in the lowest layer and were gathered in the pre-weighed glass vials. All the water-soluble components were included in the pigmented layer that was separated in methanol. In every test sample, the thick medium layer was removed.

Estimation of value of Alkaloids

Alkaloids were identified using the Harborne (1973) technique. 200 ml of 10% acetic acid in ethanol was added to 5g of the sample, covered, and left to stand for 4 hours. It was filtered and the filtrate was then concentrated to a fourth of its original volume in a water bath. Drop by drop, concentrated NH4OH was added to the filtrate until the precipitation was finished. After allowing the whole solution to settle, the precipitate was collected, cleaned with weak NH4OH, and then filtered. The alkaloid, which was dried and weighed, is the residual.

Estimation of value of Total Phenolic Compounds

The Folin-Ciocalteu reagent technique was modified to measure the total phenolic content. 7% Sodium carbonate solution in Distilled Water was added to 1 ml of plant extract, 5 ml of Folin-Ciocalteu reagent, and 5 ml of plant extract. The mixes were left to stand at room temperature for 30 minutes. Using a UV-visible spectrophotometer, the absorbance at 760 nm was measured in comparison to a blank made up of all the same ingredients as the test sample but without the test extract. Gallic acid solutions in methanol at concentrations of 1.0, 1.5, 3.0, 6.0, 12, and 24 g/ml were used to create the standard calibration curve (R2 = 0.998). Gallic Acid Equivalents (mg of GAE/g dry weight of extract), a standard reference ingredient, were used to represent the total phenolic content in the seed extract.

Estimation of value of Tannins

By using the Siddhuraj and Manian method, the amount of tannins in *H. radicata* was calculated. The extracts were divided into 500 L test tubes, each of which received 500 L of distilled water and 100 mg of polyvinyl polypyrrolidone. For four hours, this mixture was subsequently incubated at 4°C. The sample was then centrifuged for five minutes at a speed of 5000 rpm and 20 L of the supernatant was collected.

This supernatant contains no tannins and just simple phenolics (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). At 725 nm, the supernatant's phenolic content was determined and represented as the amount of free phenolic content based on dry matter. The extract's tannin content was determined using the results mentioned above in the following manner:

Total phenolics (mg GAE/g extract) minus free phenolics (mg GAE/g extract) equals tannins (mg GAE/g extract).

Ultimate analysis

Materials often consist of a diverse range of chemical compounds. To identify and quantify the chemical constituents of a specific material, a technique called "Ultimate Analysis" is employed (Sarkar et al., 2022). This method provides greater accuracy compared to "Proximate Analysis." Using this approach, a sample of the plant species was analyzed according to the standard method outlined in CEN/TS 15104:2005, which covers the determination of total carbon, hydrogen, and oxygen content in solid biofuels.

HHV

The Standard Test Method for Ash in Biomass (ASTM E1755) and the Standard Test Method for Volatile Matter in the Analysis of Particulate Wood Fuels [E872 - 82 (Reapproved 1998)] were used to explore Volatile Matter, Ash Content, Fixed Carbon and Higher heat value (MJ/kg).

FTIR

In this process, the sample is exposed to infrared radiation ranging from 10,000 to 100 cm^{-1}. Some of the

Table 1. Stock solution preparation.

Conc. of stock solution	Vol. of stock sol. Used (ml)	Vol. of dH_2O used (ml)	Conc. of working sol. (µg/ml)
10 mg/ml	10	990	10
	20	980	20
	30	970	30
	40	960	40
	50	950	50
	60	940	60
	70	930	70
	80	920	80
	90	910	90
	100	900	100

radiation is absorbed by the sample, while the rest passes through. The absorbed energy causes the sample molecules to undergo rotational or vibrational changes. The detector captures the resulting signal, typically producing a spectrum between 4000 cm^{-1} and 400 cm^{-1}, which serves as a molecular fingerprint of the sample (Bagchi et al., 2021).

Model: Thermo Fisher Scientific Nicolet TM iS10 FTIR Spectrometer, USA

GCMS

Preparation of extract

With the aid of an electric grinder, the plant's leaves were ground into a powder and sieved. Methanolic extract was prepared for further GC-MS research.

GC Programme

Model: GC-MS Model: Perkin Elmer Clarus 680 GC/ 600C MS

Oven: Initial temp 60°C for 1 min, ramp 7°C/min to 200°C, hold 3 min, ramp 10°C/min to 300°C, hold 5 min, Inj Aauto=280°C, **Volume**=0 µL, Split=10:1, **Carrier Gas**=He, Solvent Delay=8.00 min, Transfer Temp=180°C, Source Temp=150°C, **Scan**: 50 to 600Da, **Column** 60.0m x 250µm.

MS Programme

Spectrum analysis has been done using Version 2.0g of the NIST mass spectral library search programme.

Identification of the phytochemical constituents

The individual mass spectral peak values of the unidentified phytochemical components included in the methanol extract of the stem were compared with the 62,000-pattern database of the National Institute of Science and Technology. The phytochemicals were then identified based on the obtained results after contrasting the unknown peak value and chromatogram from GC-MS with the known chromatogram and peak value from the NIST Library database.

Antimicrobial test

Culture media

To cultivate *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) in a laboratory setting, it is needed to prepare specific culture media suitable for each bacterium. Here are the culture media used for growing these bacteria. Luria-Bertani (LB) Agar, Luria-Bertani Broth, Tryptic Soy Agar (TSA), and Tryptic Soy Broth (TSB) are general-purpose media suitable for the cultivation of a wide range of bacteria, including *Escherichia coli (E. coli)* and *Staphylococcus aureus* (*S. aureus*).

Bacterial strains used in the study

Escherichia coli (E. coli) and *Staphylococcus aureus* (*S. aureus*) bacterial strains were used in this study.

Plant Extract preparation:

Each plant sample was soaked in methanol and kept in shaking condition for 48 hrs. at room temperature. Then it was filtered using Whatman No-1 filter paper and the filtrates were evaporated and the crude extract was collected.

Stock solution preparation:

10 mg/ml concentration of stock solution was prepared by dissolving 0.1 g of crude extract into 10 ml of water. 10 different working conc. were prepared according to table 1. Stock solution preparation.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using a microdilution method, with nutrient broth as recommended by the NCCLS (2000). In brief, 20µL (2.5 × 10^5 CFU/mL) of each bacterial strain was added individually to 2mL of nutrient broth (NB). Different concentrations of plant extracts were added to the culture media containing the test strains. After 24 hours of incubation, the MIC values were obtained by

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checking the turbidity of the bacterial culture media. The MIC value corresponded to the concentration of particles that inhibited 99% of bacterial growth. Pure suspension of plant extract was formed by sonication and it acts as a dissolved solution which accurately reflects the amount of plant extract available in solution to act on microorganisms.

Table 2. Observed data on physical analysis.

Sample	VM	AC	FC	BD	SI
Acalypha indica	**75.452**	11.555	12.993	**0.256**	**1.456**
Physalis angulata	67.616	12.307	**15.251**	**0.223**	1.428
Senna tora	66.454	**12.327**	15.161	0.227	1.155
Euphorbia hirta	**55.289**	**10.242**	**12.365**	0.236	**1.060**
Ziziphus mauritiana	58.236	11.254	12.444	0.235	1.222

VM=Volatile Matter (%), **AC**=Ash Content (%), **FC**=Fixed Carbon (%), **BD**=Bulk Density (gm/cm3), **SI**=Swelling Index (%). *Red colour indicates Higher Values, Blue colour indicates Lower Values*.

The minimum bactericidal concentration (MBC) values of the plant extracts were determined according to the standard method (NCCLS, 2000). The MBC values were determined by subculturing the MIC dilutions onto the sterile nutrient agar plates and incubating at 37 °C for 24 hours. The lowest concentration of the extract that completely killed the tested bacteria was checked and tabulated as the MBC value. The MBC value corresponded to the concentration where 100% of the bacterial growth was arrested compared to the positive control (no treatment). All assays were performed in the bio-safety cabinet.

Table 3. Observed data on compositional analysis.

Sample	CELC	HEMC	LC	EC
Acalypha indica	**61.727**	**4.372**	4.531	1.163
Physalis angulata	60.640	3.685	5.147	**1.974**
Senna tora	61.393	**4.372**	**5.531**	1.390
Euphorbia hirta	38.453	**2.327**	4.420	**0.749**
Ziziphus mauritiana	**37.245**	3.254	**4.222**	1.235

CELC=Cellulose Content (%), **HEMC**=Hemicellulose Content (%), **LC**=Lignin Content (%), **EC**=Extractive Content (%). *Red colour indicates Higher Values, Blue colour indicates Lower Values*

Tolerance level

Bacterial tolerance levels of each strain against each plant extract were determined according to the method of May *et. al* (2006) using the following formula:

Tolerance = MBC/MIC

Disc Agar Diffusion Method

The susceptibility of different bacteria to plant extract was determined using the disc agar diffusion (DAD) technique, as described by Bauer et al. (1966). The cultured bacteria taken from an overnight culture were freshly grown for 4 hours, having 106 CFU/mL bacterial cells, which were standardized against MacFarland's standard. With this fresh bacterial culture, a bacterial lawn was prepared on Mueller-Hinton agar plate. Filter paper discs of 6-mm size were prepared by absorbing 5 µL of 12 mg/mL plant extract, used to observe susceptibility patterns. The diameter of the zone of bacterial growth inhibition surrounding the disc (including the disc) was measured as per Bauer et al. (1966).

Killing kinetic assay

Killing kinetic assay or time kill assay of *E. coli* and *S. aureus* strains was studied against five different plant extracts by the method of Guggenbichler et al. (1985). Bacterial growth after treatment (at their respective MBC values) was measured by quantifying cell viability at 0, 2, 4, 8, 12, 18 and 24 hours after incubation with plant extract. The growth inhibition percentage was obtained with respect to the positive control. Bacterial cell viability was measured spectrophotometrically using a Cistronics UV/vis spectrophotometer.

Results and Discussion

Physical analysis

Physical analysis involves analysis of proximate composition, bulk density and Swelling Index. The observed data have been represented in tabular form in Table 1 (De, 2023).

Compositional analysis

The Compositional analysis involves the percentage calculation of Cellulose, Hemicellulose, Lignin, and

Table 4. Observation on Qualitative phytochemical studies.

Phytochemicals	Results				
	Acalypha Indica	*Physalis Angulata*	*Senna tora*	*Euphorbia hirta*	*Ziziphus Mauritiana*
Carbohydrate	+	+	+	+	+
Protein	+	+	+	+	+
Total Fat	+	-	-	+	-
Alkaloids	+	+	+	+	+
Phenolics	+	+	+	+	+
Tannin	+	+	+	+	+
Saponins	-	-	-	-	-
Terpenoids	-	-	-	-	-

+= Present, -= Absent

Table 5. Observed data on quantitative phytochemical analysis.

Sample	TCH	TPR	TFA	ALKD	PHEL	TANN
Acalypha indica	**3.907**	1.201	0.330	1.132	1839.946	**0.635**
Physalis angulata	8.123	**1.040**	**0.311**	**1.261**	**2907.845**	**0.101**
Senna tora	4.761	1.932	0.591	0.801	**1281.466**	0.458
Euphorbia hirta	**9.619**	1.194	0.642	1.095	2011.451	0.330
Ziziphus mauritiana	9.142	**2.321**	**0.655**	**0.235**	2000.451	0.354

TCH =Total Carbohydrate (gm/100 gm), **TPR** =Total Protein (gm/100 gm), **TFA** =Total Fat (gm/100 gm), **ALKD** =Alkaloids (g% W/V) of dry weight), **PHEL** =Phenolics (mg/100 gm), **TANN** =Tannin (g/100g). *Red colour indicates Higher Values, Blue colour indicates Lower Values*

Table 6. Observed data on ultimate analysis.

Sample	CC	HC	OC	NC	HHV
Acalypha indica	**39.322**	6.289	32.795	1.158	16.267
Physalis angulata	42.773	6.525	32.411	**0.974**	16.269
Senna tora	**44.656**	**7.289**	30.793	1.117	15.425
Euphorbia hirta	42.600	4.560	**27.320**	**2.050**	**15.088**
Ziziphus mauritiana	43.222	**4.369**	**34.222**	0.987	**16.546**

CC=Carbon Content (%), **HC**=Hydrogen Content (%), **OC**=Oxygen Content (%), **NC**=Nitrogen Content (%), HHV=Higher Heat Value (%) *Red colour indicates Higher Values, Blue colour indicates Lower Values.*

Extractive content. The observed data have been represented in tabular form in Table 3. Observed data on compositional analysis.

Chemical and Spectroscopic Analysis

Qualitative and quantitative phytochemical studies

Preliminary phytochemical studies (Qualitative and quantitative) were performed to assess the presence of bioactive phytoconstituents. Observed data has been represented in the Table 4. Observation on Qualitative phytochemical studies.

FTIR

The FTIR graph, which is shown in Figures (4.1–4.5), showed the presence of several peaks corresponding to absorbance and wave number. Table (4.5–4.9) shows the positions of these peaks as well as information about their functional groups, chemical classes, and peak details (De, 2023).

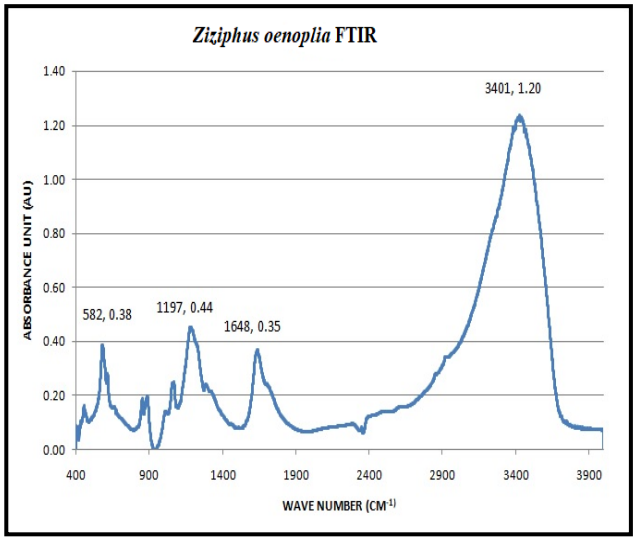

Figure 1. FTIR spectra of *Zizipus mauritiana*.

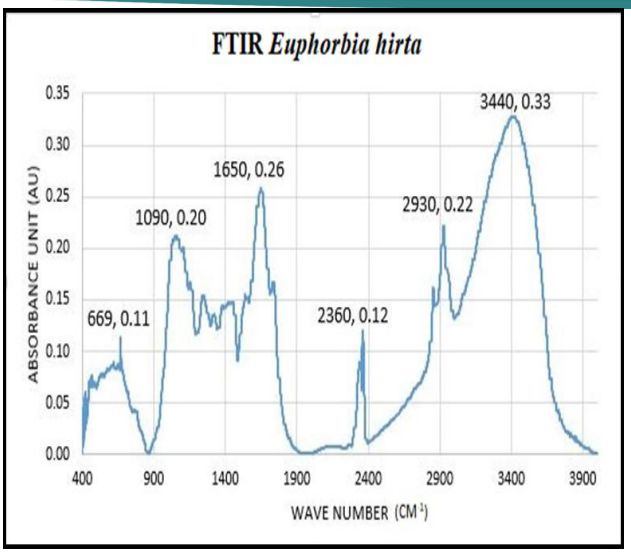

Figure 2. FTIR spectra of *Euphorbia hirta*.

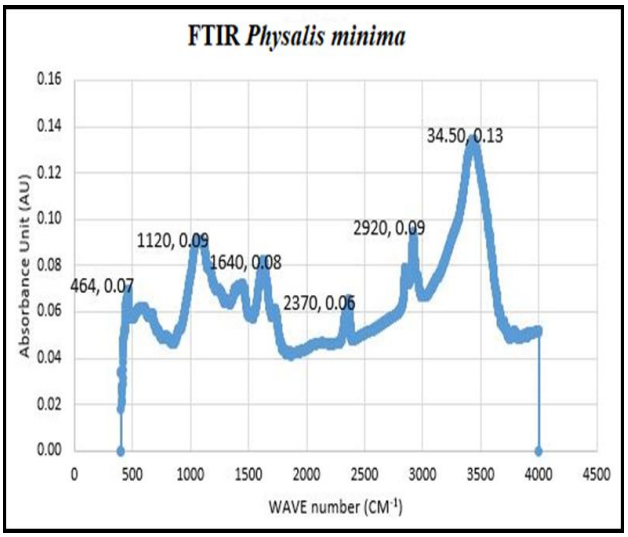

Figure 3. FTIR spectra of *Senna tora*.

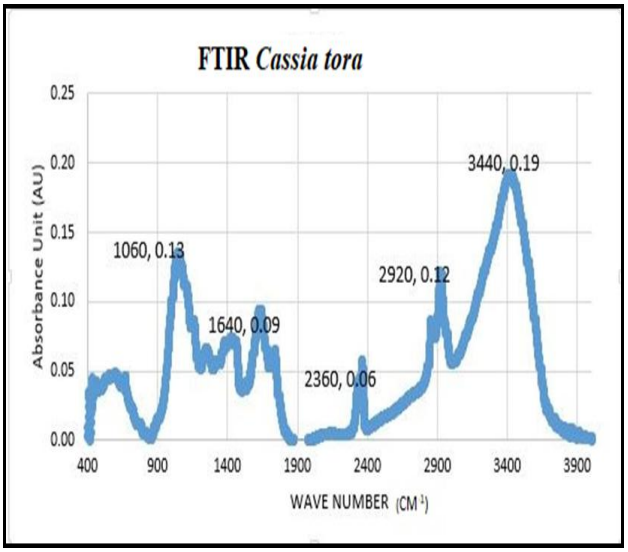

Figure 4. FTIR spectra of *Physalis angulata*.

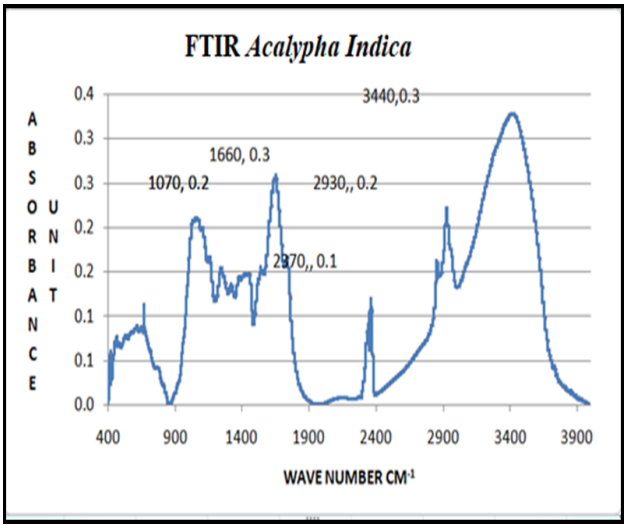

Figure 5. FTIR spectra of *Acalypha indica*.

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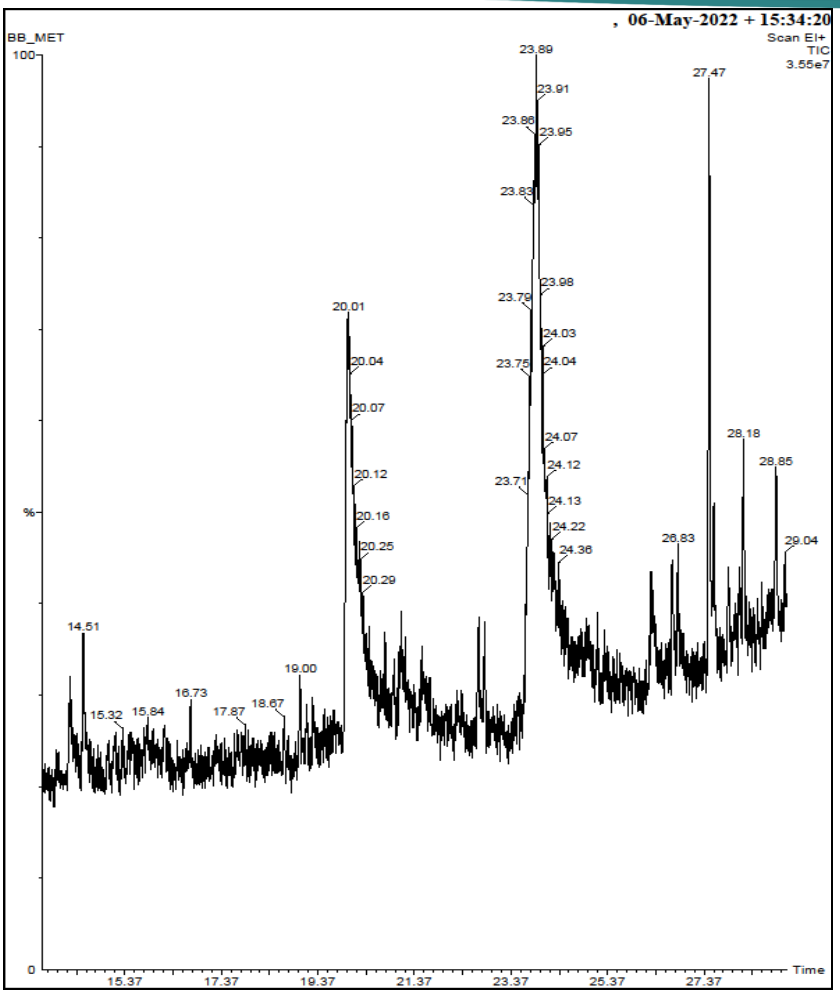

Figure 6. GC-MS Chromatogram of Methanol extract of *Acalypha indica* leaf.

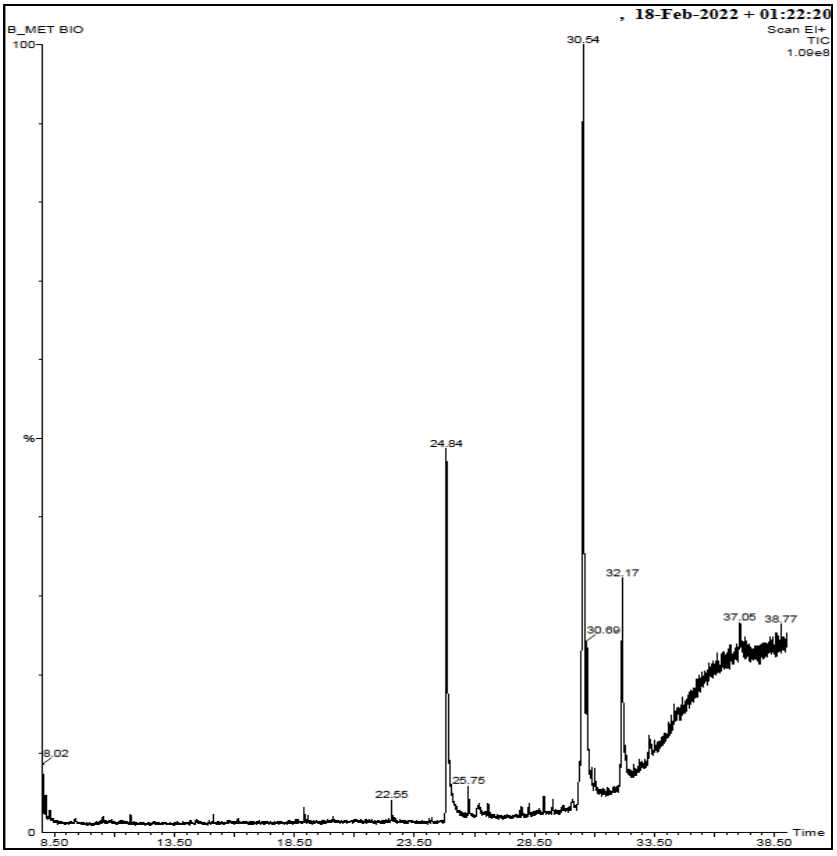

Figure 7. GC-MS Chromatogram of Methanol extract of *Senna tora* leaf.

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Table 7. Peak positions, functional groups, chemical class and peak details of leaves of five potential medicinal plants (De, 2023).

	Peak Position	Range	Group	Class	Peak Details
Ziziphus mauritiana	582	515-690	C-Br stretching	Halo Compound	Strong
	1197	1124-1205	C-O Stretching	Tertiary Alcohol	Strong
	1648	1566-1650	C=C Stretching	Cyclic Alkene	Strong
	3401	3300-3400	N-H Stretching	Aliphatic Primary Amine	Medium
Euphorbia hirta	669	665-730	C=C Bending	Alkene	Strong
	1090	1087-1124	C-O Stretching	Secondary Alcohol	Strong
	2360	2360	O=C=O Stretching	Carbon Dioxide	Strong
	2930	2840-3000	C-H Stretching	Alkene	Medium
	3440	3200-3550	O-H Stretching	Alcohol	Strong, Broad
Senna tora	1060	1050-1085	C-O Stretching	Primary Alcohol	Strong
	1640	1640-1690	C=N Stretching	Imine / Oxime	Strong
	2360	2360	O=C=O Stretching	Carbon Dioxide	Strong
	2920	2840-3000	C-H Stretching	Alkene	Medium
	3440	3200-3550	O-H Stretching	Alcohol	Strong / Broad
Physalis angulata	464	1087-1124	C-O Stretching	Secondary Alcohol	Strong
	1640	1638-1648	C=C Stretching	Alkene	Strong
	2370	2370	O=C=O Stretching	Carbon Dioxide	Strong
	2920	2840-3000	C-H Stretching	Alkane	Medium
	3450	3200-3550	O-H Stretching	Alcohol	Strong, Broad
Acalypha indica	1070	1030-1070	S=O stretching	Sulfoxide	Strong
	1660	1665-1675	C=C stretching	Alkene	Weak
	2370	2349-2370	O=C=O stretching	Carbon Dioxide	Strong
	2930	2840-3000	C-H stretching	Alkane	Medium
	3440	3200-3550	O-H stretching	Alcohol	Strong, broad

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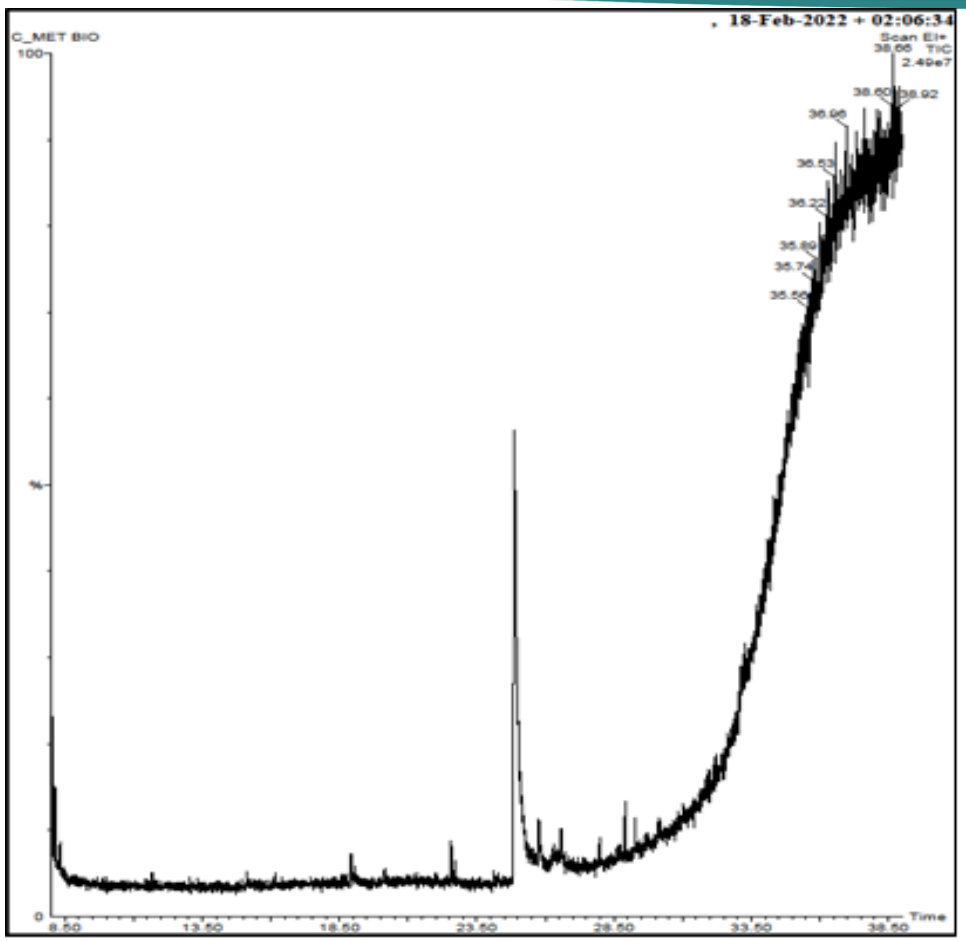

Figure 8. GC-MS Chromatogram of Methanol extract of *Euphorbia hirta* leaf.

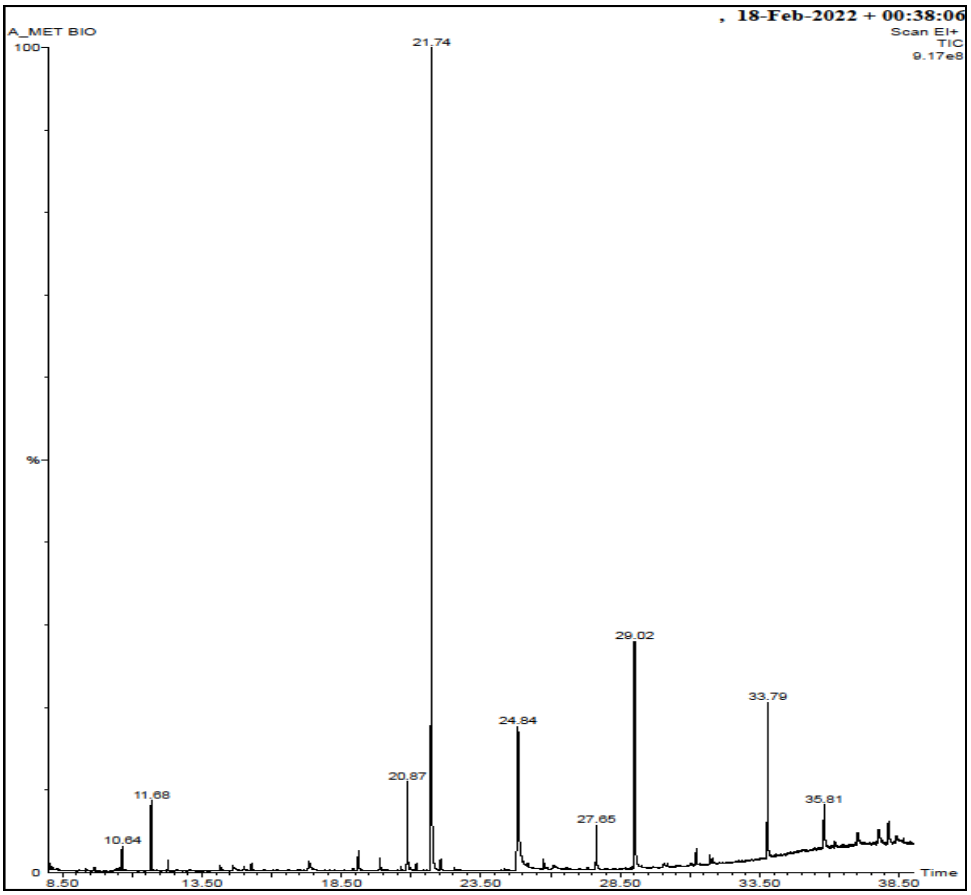

Figure 9. GC-MS Chromatogram of Methanol extract of *Physalis angulata* leaf.

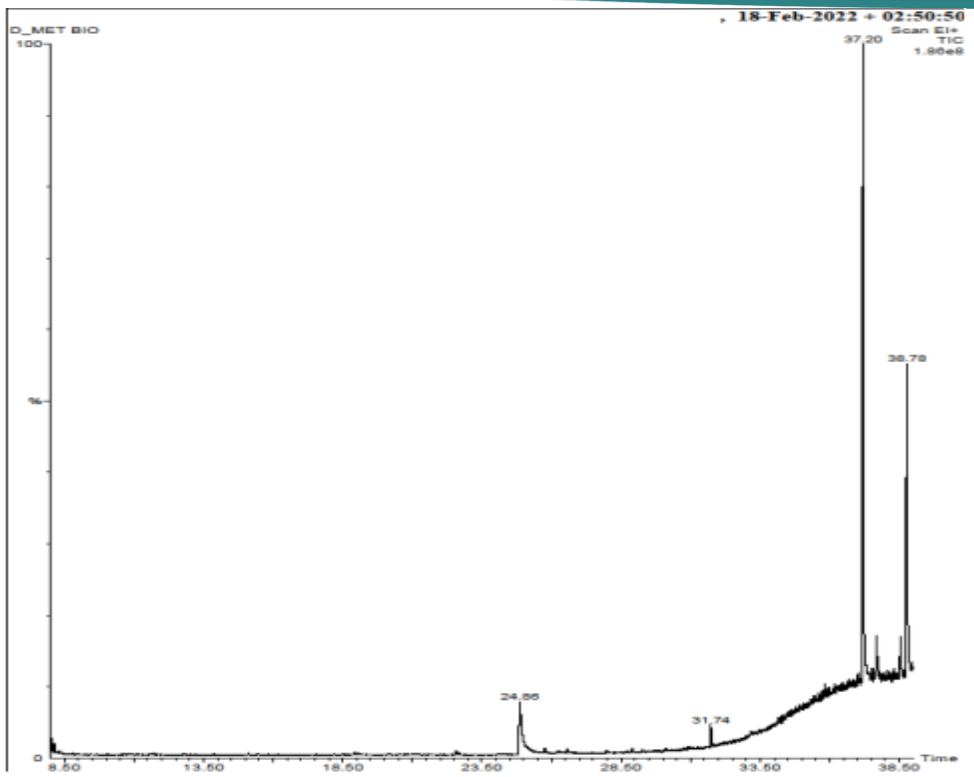

Figure 10. GC-MS Chromatogram of Methanol extract of *Ziziphus mauritiana* leaf.

Table 8. Phyto-components identified with their respective peaks, molecular weight (MW) and retention time (RT) in the methanolic extracts of Various Plants. Source: Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search/list) (De, 2023).

	Peak	RT	Compounds	MW (Da)	Bioactivity
Acalypha indica	1	35.883	CYCLOTRISILOXANE, HEXAMETHYL-	222	No record found (Novel)
	2	9.389	CIS-2,4-DIMETHYLTHIANE, S,S-DIOXIDE	162	Anticancer (stomach)
	3	14.509	1-NONADECANAMINE, N,N-DIMETHYL-	311	Antitumor (nasopharynx)
	4	20.011	TRIDECANE, 2,2,4,10,12,12-EXAMETHYL-7- (3,5,5-TRIMETHYLHEXYL)-	164	Arachidonic-Acid-Inhibitor
	5	22.697	DODECANE, 1-FLUORO-	188	No record found (Novel)
	6	23.888	MYO-INOSITOL, 4-C-METHYL	194	No record found (Novel)
	7	27.474	TETRAMETHYL-2-HEXADECEN-1-OL	296	Oligosaccharide Provider
	8	31.371	Z,Z-6,28-HEPTATRIACTONTADIEN-2-ONE	530	Increase Zinc Bioavailibility
	9	37.923	ARSENOUS ACID, TRIS(TRIMETHYLSILYL) ESTER	342	Increase Aromatic Amino Acid Decarboxylase Activity
	10	38.864	ARSENOUS ACID, TRIS(TRIMETHYLSILYL) ESTER	342	Inhibit Production of Uric Acid

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Senna tora

Peak	RT	Compounds	MW (Da)	Bioactivity
1	21.1	PALMITIC ACID	256.5	No record found (Novel)
2	25.7	ALPHA-TOCHOPHEROL	430.72	Antioxidant, Neuroprotective
3	22.73	PHYTOL OR 3,7,11,15-TETRAMETHYL-2HEXADECEN-1-OL	297	Neuroprotective, Antimicrobial, Antiasthmatic
4	16.8	LAMINITOL OR 1-METHYL1,2,3,4,5,6CYCLOHEXANEHEXOL	194.2	Anti-malarial
5	23.01	LINOLENIC ACID	278.43	Antioxidant, Neuroprotective
6	15.9	TREHALOSE	342.3	Neuroprotective
7	26.17	PHTHALIC ACID	391	No record found (Novel)
8	19.395	PHYTOL	297	Neuroprotective, Antimicrobial, Antiasthmatic
9	23.15	STEARIC ACID	284.4	Neuroprotective, cholesterol-lowering
10	26	CYCLOPENTADECANOL	226.35	No record found (Novel)

Ziziphus mauritiana

Peak	RT	Compounds	MW (Da)	Bioactivity
1	24.86	PHENOL, 3-METHOXY-2-METHYL-	138	Methyl-Guanidine-Inhibitor
2	32.2	PHENOL, 2-(2-HYDROXY-1,2-DIMETHYLCYCLOPENTYL)-, 1-ACETATE, TRANS-	248	Decrease Glutamate Pyruvate Transaminase
3	38.776	AROMADENDRENE, DEHYDRO	202	Alcohol-Dehydrogenase-Inhibitor
4	13.87	PENTADECANOIC ACID	242	Antimicrobial, antifungal, Antioxidant
5	7.26	HEPTADECANE	240	Antioxidant
6	14.07	1,2-BENZENEDICARBOXYLIC ACID, DIHEXYL ESTER	334	Reduces β-induced neurotoxicity
7	16.3	HEXADECANOIC ACID, ETHYL ESTER	284	Nematicide, Pesticide, Lubricant, Antiandrogenic
8	15.9	9-OCTADECENOIC ACID	282	Anemiagenic, anti-alopecic, choleretic, dermatitigenic

Euphorbia hirta

Peak	RT	Compounds	MW (Da)	Bioactivity
1	6.224	1,2,3- BENZENETRIOL	126	Anticancer (Lung), Antiseptic, Antioxidant, Antidermattic
2	23.888	MYO-INOSITOL, 4-C-METHYL-	194	Myo-neuro-stimulant
3	10.85	PHYTOL	296	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic
4	12.53	9- TETRADECENAL, (Z)-	210	Sex pheromone
5	14.24	13-OCTADECENAL,(Z)-	266	Antimicrobial
6	27.474	Z,Z-6,28-HEPTATRIACTONTADIEN-2-ONE	530	Increase zinc Bio-availability
7	38.864	4-TERT-OCTYLPHENOL, TMS DERIVATIVE	278	No record found (Novel)
8	9.49	HEXADECANOIC ACID, METHYLESTER	270	Antioxidant, Flavor, Hypo-cholesterolemic

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	Peak	RT	Compounds	MW (Da)	Bioactivity
Physalis Angulata	1	26.15	DODECANOIC ACID	200	Hypocholesterolemic agent
	2	18.9	DECANOIC ACID, METHYL ESTER	186	Antibacterial, Anti-inflammatory
	3	32.48	HEXADECANOIC ACID, METHYL ESTER		Antibacterial, Anti-inflammatory, Anticancer
	4	36.2	PHYTOL	296	Antioxidant, Diuretic and Anti-cancer
	5	35.78	9,12-OCTADECADIENOIC ACID (Z,Z)-,METHYL ESTER	944	Anti-cancer, Anti-inflammatory
	6	36.27	METHYL STEARATE	298	Anti-helminthic, Anti-inflammatory
	7	34.03	N-HEXADECANOIC ACID	256	Anti-bacterial, Anti-fungal
	8	33.4	DIBUTYL PHTHALATE	278	Antibacterial, Anti-inflammatory

Figure 11. Minimum inhibitory concentration of *Acalypha indica* plant extracts against *S. aureus* strains.

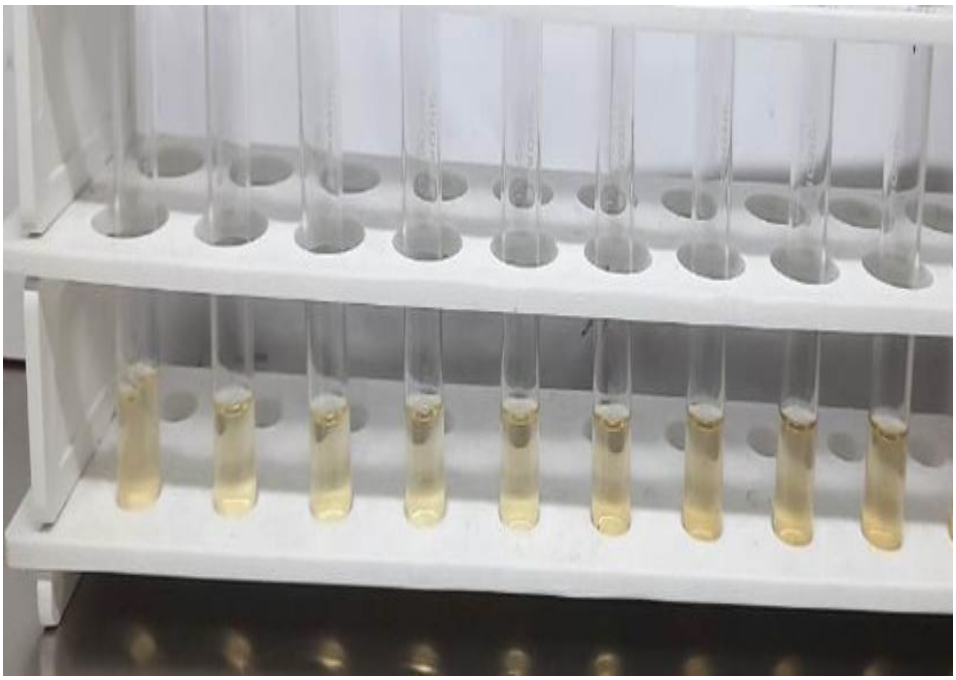

Figure 12. Minimum inhibitory concentration of *Acalypha indica* plant extracts against *E. coli* strains and *S. aureus* bacterial strains.

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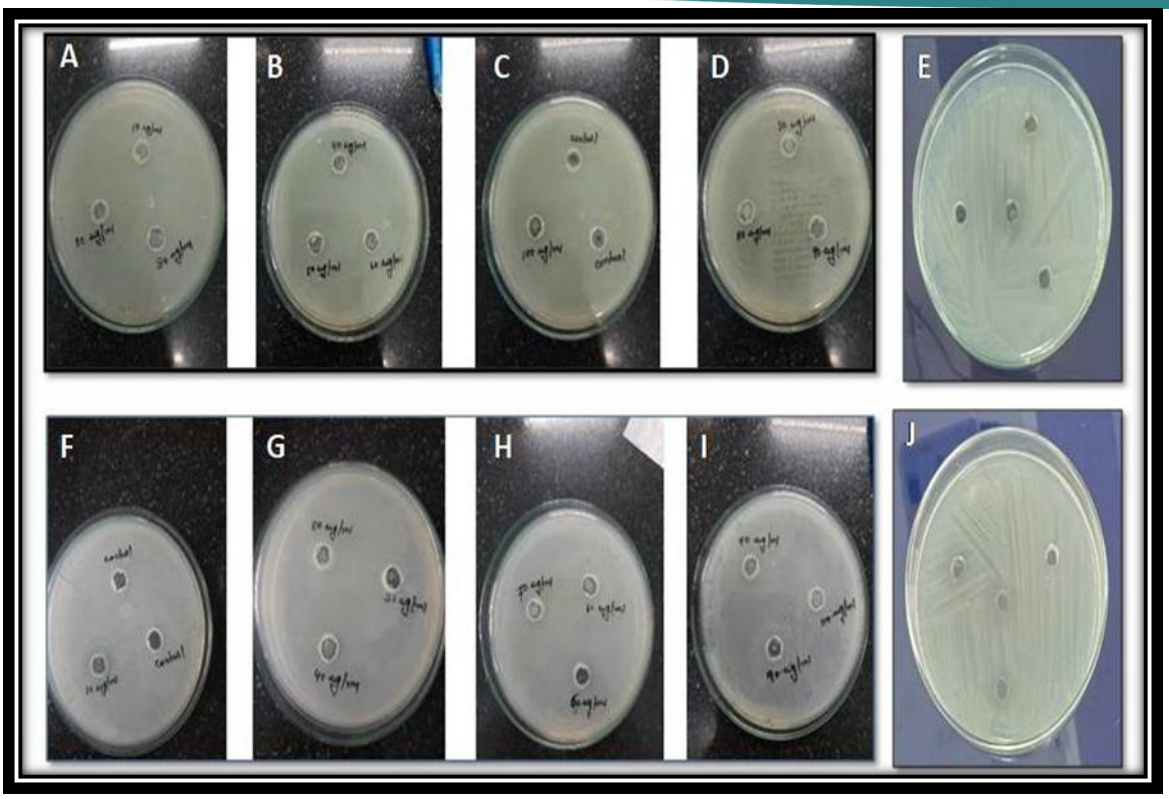

Observation on antimicrobial activity: **Tolerance level**

Figure 13. Antimicrobial activity of plant extracts was shown by the agar well diffusion method. Here, A-E: Disc agar diffusion pattern of multi-drug resistant *S. aureus* strain, and F-J: Disc agar diffusion pattern of *E. coli* strain.

Minimum Inhibitory Concentration.

Antimicrobial activity in terms of Minimum inhibitory concentration (MIC) of five different plant extract against both gram-negative *E. coli* and gram-positive *S. aureus* microorganisms at different concentrations showed that they revealed a dose-dependent antimicrobial activity against *S. aureus* microorganisms but no activities were found in *E. coli* strains. In case of S. aureus bacterial strains, growths were inhibited at 40 µg/ml in *Acalypha indica* and *Senna tora* plant extracts and growths were inhibited at 50µg/mlin *Physalis angulata, Euphorbia hirta* and *Ziziphus mauritiana* plant extracts (Fig. 4.21).

Minimum bactericidal concentration (MBC) determination.

The minimum bactericidal concentration (MBC) testing of five different plant extracts against Gram-positive *S. aureus* and Gram-negative *E. coli* showed that the extracts exhibited dose-dependent antimicrobial activity only against *S. aureus*. No inhibitory effects were observed on *E. coli* strains. For *S. aureus*, complete growth inhibition occurred at 50 µg/mL with *Acalypha indica*, while *Senna tora*, *Physalis angulata*, *Euphorbia hirta* and *Ziziphus mauritiana* required 60 µg/mL to achieve the same effect.

The tolerance level for each *S. aureus* strain was determined based on the corresponding MIC and MBC values for each plant extract. The tolerance ratio was 1.25 for *Acalypha indica*, 1.20 for *Physalis angulata*, *Euphorbia hirta*, and *Ziziphus mauritiana*, and 1.50 for *Senna tora*. Since the plant extracts showed no antimicrobial activity against *E. coli*, no tolerance values were recorded for this strain.

Disc agar Diffusion (DAD)

The zones of inhibition of *E. coli* and *S. aureus* against 5 different plant extracts, standard antibiotic as positive control, and distilled water as negative control are shown in Table 4.1 and Figure 4.21. No zones were found in *E. coli* strains against all the plant extracts. Zones of 11±0.6 mm, 8±0.6 mm, 10±0.4 mm, 8±0.8, and 9±0.8 mm were observed in S. aureus strain on their respective MIC and MBC doses.

Killing kinetic assay

The time-kill curve of plant extracts against *S. aureus* strains is presented in Figure 4.22. Bactericidal activity was gradually increased to 8 hours of exposure of the bacteria against plant extracts at their respective MBC concentrations for S. aureus strains, and all bacteria were killed within this period. In our study, all plant extracts were effective in inhibiting bacterial growth in a dose and time-dependent manner in *S. aureus* bacteria.

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Table 9. % of viable cells of *S. aureus* bacteria at different times in different plant extract treatments.

Treatment Time	% of viable *S. aureus* cells				
	Acalypha indica	*Senna tora*	*Physalis angulata*	*Euphorbia hirta*	*Ziziphus mauritiana*
0 hrs	5.35±0.42	6.28±0.48	5.94±0.48	7.28±1.08	6.38±0.64
1 hrs	59.00±2.59	42.68±2.35	58.28±2.65	51.32±2.83	68.25±4.25
4 hrs	62.35±3.46	53.59±2.69	69.25±3.58	58.24±3.25	71.05±3.82
8hrs	24.32±1.58	15.29±2.46	23.58±1.58	19.58±1.97	26.47±1.29
12hrs	14.35±0.78	8.25±1.25	12.58±1.95	18.29±1.25	16.54±1.27
16hrs	5.48±0.59	7.58±0.95	8.25±1.20	9.18±1.95	7.58±1.08
20hrs	5.05±1.04	6.25±0.46	5.28±0.58	6.23±0.93	5.94±0.68
24hrs	6.36±1.42	7.58±0.87	6.18±0.67	6.84±0.59	6.25±0.54

Analysis

Physical analysis

The percentage composition of volatile matter was highest in *Acalypha indica* (75.452%) whereas it was lowest in *Euphorbia hirta* (55.289%). The percentage composition of Ash content was highest in *Senna tora* (12.327%), whereas it was lowest in *Euphorbia hirta* (10.242%). The percentage composition of Fixed carbon was highest in *Physalis angulata* (15.251%), whereas it was lowest in *Euphorbia hirta* (12.365%). The percentage composition of Bulk Density was highest in *Acalypha indica* (0.256%), whereas it was lowest in *Physalis angulata* (0.223%). The percentage composition of the Swelling Index was highest in *Acalypha indica* (1.456%), whereas it was lowest in *Euphorbia hirta* (1.222%) (De, 2023).

Compositional analysis

The percentage composition of Cellulose Content was highest in *Acalypha indica* (61.727%) whereas it was lowest in *Ziziphus mauritiana* (37.245%). The percentage composition of Hemicellulose Content was highest in *Acalypha indica* (4.372%) and *Senna tora* (4.372%), whereas it was lowest in *Euphorbia hirta* (2.327%). The percentage composition of Lignin Content was highest in *Senna tora* (5.531%), whereas it was lowest in *Ziziphus mauritiana* (4.222%). The percentage composition of Extractive Content was highest in *Physalis angulata* (1.974%), whereas it was lowest in *Euphorbia hirta* (0.749%) (De, 2023).

Chemical and Spectroscopic Analysis

The highest concentration of Total Carbohydrate has been observed to occur in *Euphorbia hirta* (9.619 gm/100 gm), whereas it was lowest in *Acalypha indica* (3.907 gm/100 gm). The highest concentration of Total Protein has been observed to occur in *Ziziphus mauritiana* (2.321gm/100 gm), whereas it was lowest in *Physalis angulata* (1.040 gm/100 gm). The highest concentration of Total Fat has been observed to occur in *Ziziphus mauritiana* (0.655 gm/100 gm), whereas it was lowest in *Physalis angulata* (0.311 gm/100 gm). The highest concentration of Alkaloids has been observed to occur in *Physalis angulata* (1.261 gm/100 gm), whereas it was lowest in *Ziziphus mauritiana* (0.235gm/100 gm). The highest concentration of Phenolics has been observed to occur in *Physalis angulata* (2907.845 gm/100 gm), whereas it was lowest in *Senna tora* (1281.466 gm/100 gm). The highest concentration of Tannin has been observed to occur in *Acalypha indica* (0.635 gm/100 gm), whereas it was lowest in *Physalis angulata* (0.101 gm/100 gm).

The percentage composition of Carbon Content was highest in *Senna tora* (44.656%), whereas it was lowest in *Acalypha indica* (39.322%). The percentage composition of Hydrogen Content was highest in *Senna tora* (7.289%), whereas it was lowest in *Ziziphus mauritiana* (4.369%). The percentage composition of Oxygen Content was highest in *Ziziphus mauritiana* (34.222%), whereas it was lowest in *Euphorbia hirta* (27.320%). The percentage composition of Nitrogen

Content was highest in *Euphorbia hirta* (2.050%), whereas it was lowest in *Physalis angulata* (0.974%). The percentage composition of Higher Heat Value was highest in *Ziziphus mauritiana* (1.456%), whereas it was lowest in *Euphorbia hirta* (15.088%).

FTIR (Fourier Transform Infra-red spectroscopy) of the leaves of five plant samples was conducted to determine the presence of functional groups and understand the significance of particular bioactive components. The values of peaks and functional groups were depicted in Figures (6-10) and Table 6, respectively.

The presence of functional groups like Halo Compound, Tertiary Alcohol, Cyclic Alkene, and Aliphatic Primary Amine in the leaves of *Ziziphus mauritiana* was confirmed by the more intense absorption band occurring at 582, 1197, 1648, and 3401 cm-1 corresponding to C-Br stretching, C-O stretching, C=C Stretching, and N-H Stretching, bending, and vibrations, respectively.

The presence of functional groups like Alkene, Secondary Alcohol, Carbon Dioxide, Alkene and Alcohol in the leaves of *Euphorbia hirta* was confirmed by the more intense absorption band occurring at 669, 1090, 2360, 2930 and 3440 cm^{-1} corresponding to C=C Bending, C-O Stretching, O=C=O Stretching, C-H Stretching and O-H Stretching, bending, vibrations respectively.

The presence of functional groups like Primary Alcohol, Imine / Oxime, Carbon Dioxide, Alkene and Alcohol in the leaves of *Senna tora* was confirmed by the more intense absorption band occurring at 1060, 1640, 2360, 2920 and 3440 cm^{-1} corresponding to C-O Stretching C=N Stretching, O=C=O Stretching, C-H Stretching and O-H Stretching, bending, vibrations respectively.

The presence of functional groups like Secondary Alcohol, Alkene, Carbon Dioxide, Alkane and Alcohol in the leaves of *Physalis angulata* was confirmed by the more intense absorption band occurring at 464, 1640, 2370, 2920 and 3450cm^{-1} corresponding to C-O Stretching, C=C Stretching, O=C=O Stretching, C-H Stretching, O-H Stretching, bending, vibrations, respectively.

The presence of functional groups like Sulfoxide, Alkene, Carbon Dioxide, Alkane and Alcohol in the leaves of *Acalypha indica* was confirmed by the more intense absorption band occurring at 1070, 1660, 2370, 2930 and 3440 cm^{-1} corresponding to S=O stretching, C=C stretching, O=C=O stretching, C-H stretching and O-H stretching, bending, vibrations respectively.

GC-MS (Gas Chromatography-Mass Spectroscopy) reveals the presence of important bioactive compounds in five different medicinal plants.

A total of 10 (*Acalypha indica, Senna tora*), 8 (*Ziziphus mauritiana, Euphorbia hirta, Physalis Angulata*) bioactive compounds were obtained from the peaks of methanolic extracts of five different medicinal plants by GC-MS analysis. The details of the bioactive compounds, their retention time, molecular weight and bioactivity were tabulated (Table 7). Figure (11-15) shows the chromatogram of the compounds detected using GC-MS. The bioactive compounds like Cyclotrisiloxane, Hexamethyl-, Dodecane, 1-Fluoro-, Myo-inositol, 4-C-Methyl obtained from *Acalypha Indica* leaf extract, palmitic acid, phthalic acid, Cyclopentadecanol obtained from *Senna tora* leaf extract and 4-tert-octylphenol, TMS Derivative obtained from *Euphorbia hirta* leaf extract have no records of bioactivity as per Dr. Duke's ethnobotanical and pharmaceutical database and are considered as novel bioactive compounds (De, 2023).

Study of antimicrobial activity

Determination of MIC and MBC

The five plant extracts tested displayed strong, dose-dependent antibacterial activity against the Gram-positive *S. aureus*, but showed no inhibitory effect on the Gram-negative *E. coli*. In all cases, increasing the concentration of the extracts led to a reduction in *S. aureus* growth, confirming their effectiveness against Gram-positive bacteria only. The MIC for *Acalypha indica* and *Senna tora* was determined to be 40 µg/mL, while *Physalis angulata*, *Euphorbia hirta* and *Ziziphus mauritiana* exhibited MICs of 50 µg/mL.

To accurately determine MBC values and eliminate errors caused by turbidity from insoluble substances in the broth, MIC dilutions were subcultured onto sterile Mueller Hinton agar plates. The MBC was identified as the lowest concentration at which no visible bacterial growth was observed. For *S. aureus*, *Acalypha indica* had an MBC of 50 µg/mL, while the remaining extracts showed MBCs of 60 µg/mL. These results suggest that the extracts penetrate bacterial cells, first exerting a bacteriostatic effect before achieving bactericidal action.

The lack of activity against *E. coli* is attributed to its outer membrane, which acts as a selective barrier. Unlike Gram-positive bacteria, Gram-negative organisms possess an outer membrane separate from the peptidoglycan layer. This membrane protects cells from harmful agents such as antibiotics, toxins, and enzymes, while allowing essential nutrients to enter. In *E. coli*, the outer membrane's lipid bilayer is asymmetrical—

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composed of lipopolysaccharides (LPS) in the outer leaflet and densely packed phospholipids in the inner leaflet. Studies, including those by Amro et al. (2000), have shown that the LPS layer plays a key role in restricting the entry of antimicrobial agents, explaining the resistance observed in Gram-negative bacteria.

Determination of Tolerance

The corresponding MIC and MBC values were used to calculate each strain's level of tolerance to plant extracts. When charged with *Acalypha indica*, the tolerance threshold for the *S. aureus* strain was 1.25, whereas when charged with *Physalis angulata, Euphorbia hirta* and *Ziziphus mauritiana* it was 1.20 and when charged with *Senna tora* it was 1.50 for the *S. aureus* strain. The consequence is that while bacteriostatic drugs just prevent bacterial development, bactericidal agents really kill microorganisms.

The antimicrobial agent is regarded as bacteriostatic when the MBC/MIC ratio for bacteria is more than or equal to 16 and bactericidal when it is less than or equal to 4 (Woods and Washington, 1995).

Disk agar diffusion (DAD)

This was clear from the measurements of the zone of inhibition's diameter that were taken during the evaluation of antibacterial activity. The zones of inhibition of *E. coli* and *S. aureus* against 5 different plant extracts, standard antibiotic as positive control, and distilled water as negative control are shown in Table 4.1 and Figure 4.21. No zones were found in *E. coli* strains against all the plant extracts. Zones of 11±0.6 mm, 8±0.6 mm, 10±0.4 mm, 8±0.8, and 9±0.8 mm were observed in *S. aureus* strain on their respective MIC and MBC doses.

Table 10. MIC, MBC, DAD values of Plant extracts against *S. aureus* strains.

Antimicrobial activity of 5 different plant extracts against *S. aureus* strains				
Plant extracts	**MIC (µg/ml)**	**MBC(µg/ml)**	**Tolarance (MBC/MIC ratio)**	**DAD (mm) (MBC doses)**
Acalypha indica	40 µg/ml	50 µg/ml	1.25	11±0.6 mm
Physalis angulata	50 µg/ml	60 µg/ml	1.20	8±0.6 mm
Senna tora	40 µg/ml	60 µg/ml	1.50	10±0.4 mm
Euphorbia hirta	50 µg/ml	60 µg/ml	1.20	8±0.8mm
Ziziphus mauritiana	50 µg/ml	60 µg/ml	1.20	9±0.8 mm

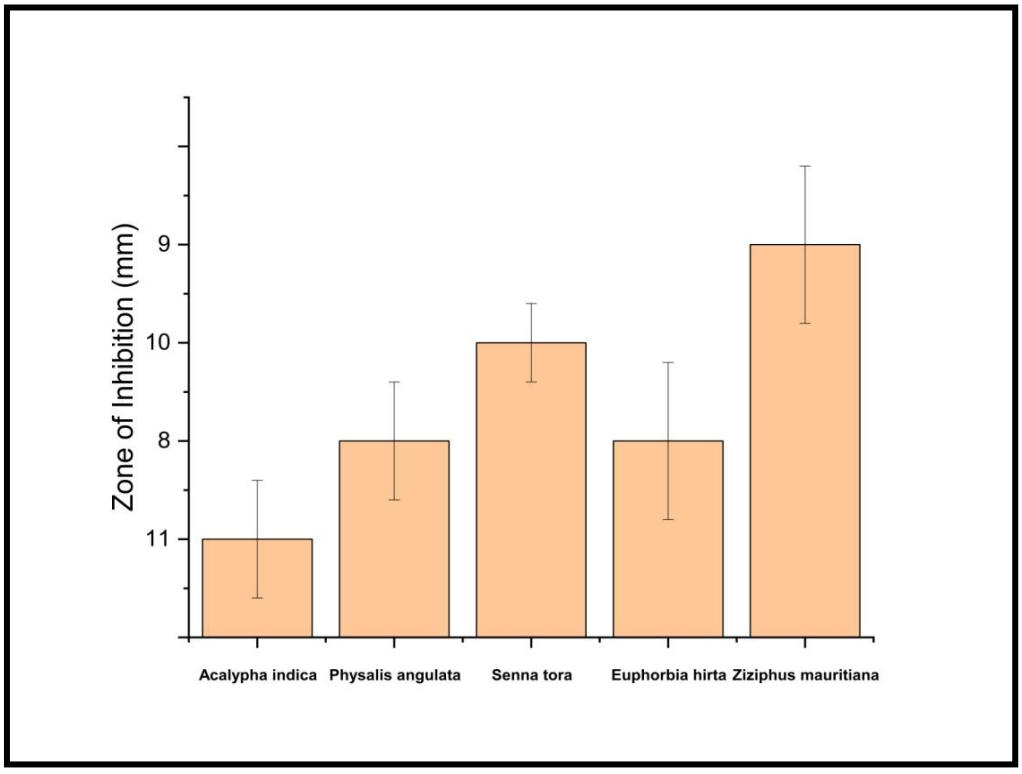

Figure 14. DAD of all Experimental plants.

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Killing Kinetic Assay

As there was no significant variation in the bactericidal effects of the five plant extracts on *S. aureus*, a killing kinetic assay was conducted to determine the minimum time required to achieve bactericidal activity. Figure XX illustrates the time-kill curves for each extract against *S. aureus* strains. Bacterial exposure to the extracts at their respective MBCs was extended up to 8 hours, resulting in complete bacterial eradication. All extracts demonstrated rapid, time-dependent bactericidal effects, causing the bacteria to enter an early stationary phase. The findings confirm that the plant extracts inhibited bacterial growth effectively in both a dose- and time-dependent manner, with total bacterial elimination observed after 8 hours of treatment.

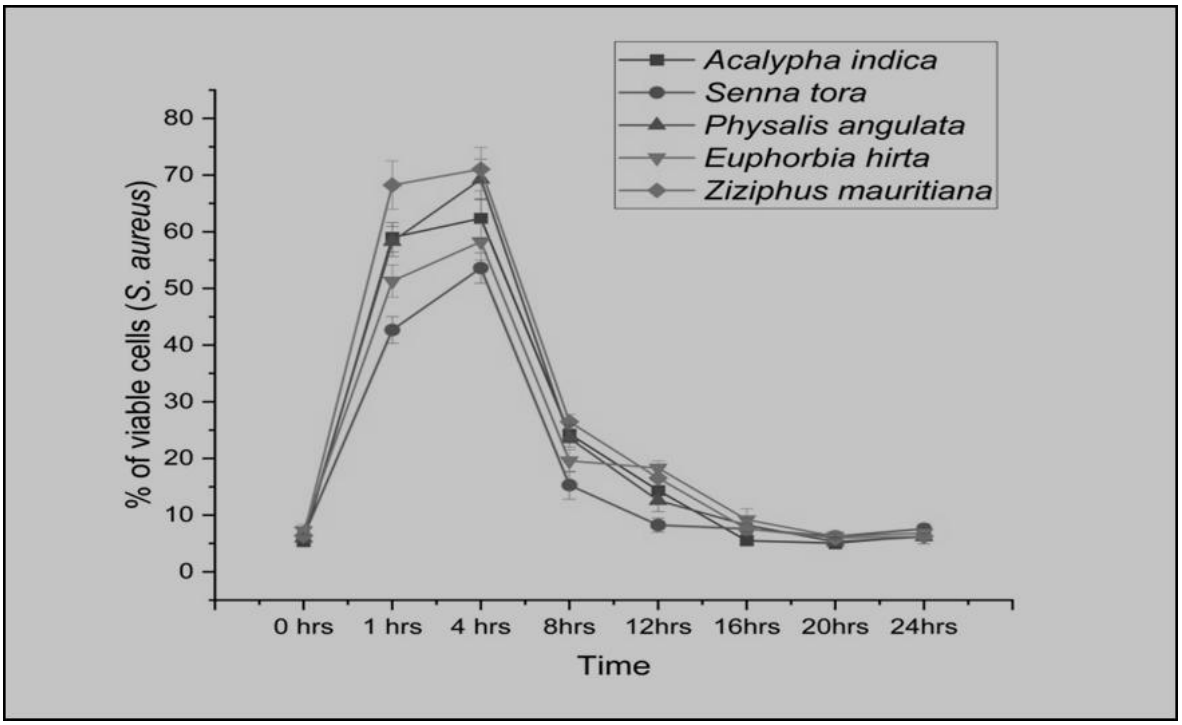

Figure 15. Killing Kinetic Assay of all plants.

Conclusion

Medicinal plants are the 'gift of nature' as they contain important bioactive chemical compounds *Acalypha indica, Senna tora, Euphorbia hirta, Physalis angulata* and *Ziziphus mauritiana* were chosen as the five medicinal plants for physico-chemical and spectroscopic characterization in order to assess bioactive potential and to support traditional beliefs in the context of scientific interpretation. Fresh leaves from the Paschim Medinipur district of West Bengal, India, were collected, dried, and pulverized for proximate, ultimate, and compositional analyses, as well as FTIR and GC-MS. The study found that compared to other experimental plants, *Acalypha indica* leaves had the highest percentage of volatile matter (%), bulk density (%), swelling index (%), cellulose content (%), hemicellulose content (%) and tannins (g/100g). The percentage composition of fixed carbon and extractive content in *Physalis angulata* was higher than that in other species. Ash, hemicellulose, and lignin contents were observed to be higher in the *Senna tora* leaf than in others. In comparison to other plants, *Euphorbia hirta* had the greatest quantities of total carbohydrates and nitrogen. *Ziziphus mauritiana* had higher protein, fat, oxygen content, and heat values than other plants. FTIR (Fourier Transform Infrared Spectroscopy) was used to analyse the leaves of five different plant samples to identify the presence of functional groups and the significance of the presence of a particular bioactive component.

GC-MS (Gas Chromatography Mass Spectroscopy) has revealed the presence of major bioactive compounds in five different medicinal plants. Five different medicinal plants' methanolic extracts were examined using GC-MS analysis and a total of 10 bioactive compounds (*Acalypha indica, Senna tora*) and 8 bioactive compounds (*Ziziphus mauritiana*, *Euphorbia hirta, Physalis Angulata*) were identified.

The results of this study demonstrate that plant extracts from *Acalypha indica*, *Senna tora*, *Physalis angulata*, *Euphorbia hirta* and *Ziziphus mauritiana* exhibit significant antibacterial activity against *Staphylococcus aureus*, a Gram-positive bacterium, while showing no inhibitory effect against *Escherichia coli*, a

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Gram-negative bacterium. The susceptibility of *S. aureus* to plant extracts, as indicated by the MIC, MBC and DAD assays, strongly suggests that these extracts contain bioactive compounds capable of penetrating and disrupting bacterial cell structures. However, the absence of antimicrobial activity against *E. coli* supports the well-established concept that Gram-negative bacteria have an outer membrane composed of lipopolysaccharides (LPS), which acts as a selective permeability barrier, preventing many antimicrobial agents from entering the cell.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC results show that *Acalypha indica* and *Senna tora* are the most potent antimicrobial agents against *S. aureus*, with MICs of 40 µg/mL and MBCs of 50 µg/mL and 60 µg/mL, respectively. The other extracts—*Physalis angulata*, *Euphorbia hirta* and *Ziziphus mauritiana*—had slightly higher MICs (50 µg/mL) and MBCs (60 µg/mL), indicating lower potency. These findings align with previous studies attributing the strong antibacterial effects of *Acalypha indica* and *Senna tora* to their rich content of flavonoids, alkaloids, and anthraquinones, while the other plants exhibit moderate antimicrobial activity.

Tolerance (MBC/MIC Ratio) and Bactericidal vs. Bacteriostatic Effects

The MBC/MIC ratios of the plant extracts ranged from 1.20 to 1.50, indicating bactericidal activity against *S. aureus*. *Senna tora* showed the highest ratio (1.50), suggesting it has a stronger initial bacteriostatic effect. Other extracts, such as *Physalis angulata*, *Euphorbia hirta*, and *Ziziphus mauritiana*, had lower ratios (1.20), confirming their bactericidal nature. These findings align with established criteria, where an MBC/MIC ratio ≤4 indicates bactericidal activity, as noted by Woods (1995) and Parekh & Chanda (2007).

Disk Agar Diffusion (DAD) and Zone of Inhibition

The Disk Agar Diffusion (DAD) assay confirmed the antimicrobial activity of the plant extracts against *S. aureus*, with inhibition zones ranging from 8 to 11 mm. *Acalypha indica* showed the highest activity (11±0.6 mm), while others had moderate effects. These results support previous findings that plant compounds like flavonoids, tannins, and alkaloids contribute to bacterial inhibition. No activity was observed against *E. coli*, likely due to its outer membrane acting as a barrier to hydrophobic plant compounds, as noted by Amro et al. (2000).

Killing Kinetic Assay and Time-Dependent Bactericidal Activity

The time-kill curve analysis showed that all five plant extracts had time-dependent bactericidal effects against *S. aureus*, completely eliminating the bacteria within 8 hours at MBC concentrations. This suggests a dose- and time-dependent antibacterial mechanism, possibly involving cell wall disruption, protein synthesis inhibition, or metabolic interference. These findings align with previous studies, such as those by Sudha et al. (2011) and Memon et al. (2010), which also reported time-dependent antibacterial activity of plant extracts.

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Conflict of interest

None

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